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Review

The histone LSD1 demethylase in stemness and cancer transcription programs [☆]

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ABSTRACT

DNA and histone chromatin modifying enzymes play a crucial role in chromatin remodeling in several biological processes. Lysine-specific demethylase 1 (LSD1), the first identified histone demethylase, is a relevant player in the regulation of a broad spectrum of biological processes including development, cellular differentiation, embryonic pluripotency and cancer. Here, we review recent insights on the role of LSD1 activity in chromatin regulatory complexes, its functional role in the epigenetic changes during embryonic development, in the establishment and maintenance of stemness and during cancer progression.

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1. Introduction

Epigenetic modifications are essential for normal development and maintenance of tissue specific patterns of gene expression. Their heritability is mediated by epigenetic modifications such as methylation of cytosine in DNA, histone posttranslational modifications, and nucleosome positioning along the DNA. Disruption of each one of these epigenetic processes can lead to altered gene functions and malignant cellular transformation in cancers. Drastic changes in the cellular epigenetic profile of somatic cells occur during differentiation of cell identity, while disruption of their epigenetic signatures by reprogramming factors may lead to the acquisition of cell pluripotency [1–4].

Widespread epigenetic reprogramming occurs either during stem cell differentiation and malignant transformation; genes involved in differentiation programs are characterized by a poised transcriptional status maintained by specific histone methylation marks typical of transcriptional activation (H3K4me3) or repression (H3K27me3) at their regulatory regions [3]. Among histone modifications, methylation and its effects on gene expression are recently gaining popularity since it was found that methylation marks are not static but dynamically regulated by histone methyltransferases and histone demethylases [5,6]. Many studies have recently shed light on the role of the lysine specific demethylase LSD1 (also known as KIAA061/KDM1/AOF2) in the

maintenance of the pluripotency in stem cells and in the oncogenic gene programs that determine a block of cell differentiation [7–11]. The role of LSD1 in gene expression is emerging in the last few years because our understanding of epigenetic complexity and plasticity of genomes is exponentially growing up following the development of new technologies that are providing comprehensive maps of localization of histone modifications.

In this review we focus on the emerging evidences that correlate LSD1 activity with epigenetic changes in the biology of stem and cancer cells.

2. LSD1 structure and function

A protein homologous to FAD-dependent oxidoreductases, KIAA061 (LSD1) was found in the HDAC1 complexes [12] although the function was not known at that time. Studies from Yang Shi's group reported the identification of KIAA061 (LSD1) in the CtBP complex [13] leading to the discovery of the first histone demethylase in 2004 [14]. Subsequent studies have shown histone demethylase activity for the closely related KDM1B/AOF1/LSD2, as well as for orthologs in other species [15–17].

LSD1 is a flavin-containing amino oxidase (AO) that specifically catalyzes the demethylation of mono- and di-methylated histone H3 lysine 4 [14,18–22] through a FAD-dependent oxidative reaction (Fig. 1A). In this reaction, FAD oxidizes the methyl-lysine generating an imine intermediate that is subsequently hydrolyzed to form unmodified lysine and formaldehyde while the reduced FAD is re-oxidized by oxygen. LSD1 demethylates mono-/di-methylated lysines, but not tri-methylated ones [14,18–22]. LSD1 is highly conserved and contains three protein domains: an N-terminal SWIRM (Swi3p/Rsc8p/Moira) structural domain, a central

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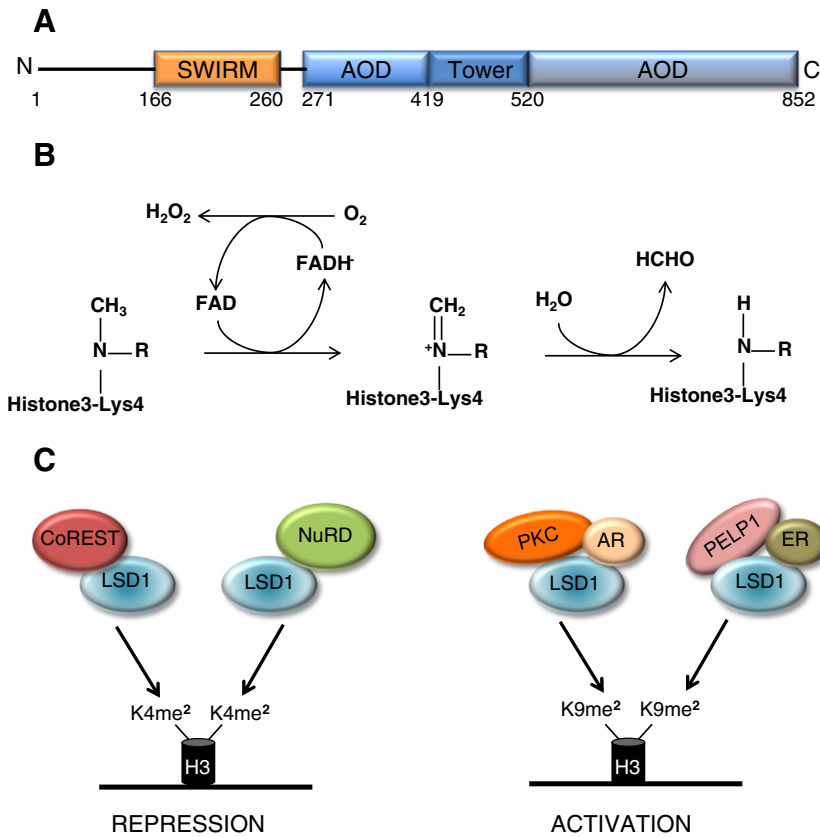


Fig. 1. A. Schematic representation of the LSD1 protein. B. FAD-dependent demethylation of dimethylated lysine residues by LSD1. C. LSD1-associated complexes and their demethylation targets.

protruding tower domain and a C-terminal amine oxidase (AO) domain [22]. The SWIRM and AO domains interact to form a core structure that binds FAD not covalently and serves as the enzymatic domain; the tower domain provides a surface platform for interaction with partners (Fig. 1B). Because of the strong structural and mechanistic similarities between LSD1 catalytic domain and conventional amine oxidases, monoamine oxidase (MAO) inhibitors as the tranlylcyproline (TCP), covalently bind FAD and inhibit LSD1 activity [23]. On the other hand, pargyline, a propargylamine containing small molecule initially proposed as LSD1 inhibitor, failed to appreciably inactivate LSD1 in subsequent studies [21,24,25]. A series of new tranlylcyproline analogs have been developed and biochemical and biological evaluation of their inhibitory properties and efficacy for human LSD1 and LSD2 has been assayed [23–25]. Some of these compounds are effective LSD1 inhibitors, and most importantly they exhibit *in vivo* efficacy in tumors by altering the chromatin state and synergistically cooperate with antitumoral drugs [24,25].

The molecular mechanism underlying LSD1 transcriptional regulation remains confuse, essentially because LSD1 associates with different complexes and it can function as co-repressor or co-activator in a target-specific manner. LSD1 has been found in different transcriptional complexes involved in transcription repression such as CoREST and NuRD (Fig. 1C) [13]. Consistent with its role in transcription repression, LSD1 demethylates monomethyl and dimethyl histone H3 lysine 4 (H3K4me1 and H3K4me2), which are marks of active chromatin transcription state.

LSD1 has also been found to have a role in transcriptional activation as exemplified by the nuclear hormone receptors induced transcription. The interaction of LSD1 with androgen (AR) or estrogen (ER) nuclear receptors seems to change its substrate specificity from H3K4me1/me2 to H3K9me1/me2 [26–31]; moreover, it has been recently reported that, following hormone treatment, protein kinase C is recruited to AR

target promoters and phosphorylates H3 threonine 6 (H3T6). This modification switches LSD1 H3K4 demethylating activity from H3K4me2 to H3K9me1 and H3K9me2 [27]. Also, ER mediated demethylation of H3K9 by LSD1 has been hypothesized to be due to H3K9 demethylase associated with LSD1 or to a reader of H3 methylation that changes LSD1 specificity [31]. As demethylation reaction by LSD1 release H_2O_2 , it has been proposed that LSD1 recruitment by ER and Myc on their respective targets, triggers DNA oxidation and recruitment of base excision repair enzymes that favors chromatin looping for transcriptional activation–repression [28,32,33]. More recently, LSD1 was found to be part of protein complexes responsible for transcription elongation: the ELL complex containing the P-TEFb transcriptional elongation factor and the MLL super-complex containing both transcriptional activators and repressors [34,35].

It is evident that association of LSD1 with specific partners determines its substrate specificity. Moreover, concomitant histone modifications such as deacetylation or phosphorylation may influence LSD1 activity as H3K9 acetylation and/or H3S10 phosphorylation negatively affect LSD1 H3K4 demethylase activity [11,21,24,26].

3. Non-histone LSD1 substrates

It has been found that LSD1 demethylates also non-histone proteins. LSD1 specifically demethylates p53 dimethylated K370 residue, significantly altering its function [36,37], indeed, dimethylated but not monomethylated p53 can interact with its coactivator 53BP1. These studies point to an active involvement of LSD1 in the DNA damage response pathway, via direct modulation of p53 activity, and suggest that LSD1 may inhibit apoptosis. LSD1 also inhibits DNA damage-induced cell death in the absence of p53 through modulation of the E2F protein stability [38,39]. Demethylation of E2F1 in lysine185 inhibits other E2F modifications that drive E2F degradation thus, favoring E2F accumulation

following DNA damage. LSD1 interacts with and demethylates the myosin phosphatase MYPT1, a phosphatase involved in Rb dephosphorylation [40]. MYPT1 demethylation enhances its ubiquitination and instability; consequently, MYPT1 degradation enhances Rb phosphorylation and may affect cell cycle consistent with high levels of LSD1 and low levels of MYPT1 found in cancer.

Among the LSD1 substrates there is also Dnmt1 [41], the major enzyme responsible for maintaining DNA methylation during DNA replication. LSD1 knock out in mouse embryonic stem (ES) cells leads to decrease in Dnmt1 protein levels and a concomitant loss of global DNA methylation. These studies revealed that LSD1, in addition to its direct activity on chromatin structure, also regulates global or specific gene expression patterns through demethylation of non-histone targets.

4. Developmental role of LSD1

Genetic studies in multiple model systems have shown that LSD1 is an important player in developmental processes [9]. In mice loss of LSD1 causes embryonic lethality at approximately day 6 [41,42]. LSD1 expression is restricted to epiblast and LSD1-null mice embryos are reduced in size compared to heterozygous controls suggesting a block to development shortly after implantation [41,42]. Embryonic lethality in these mice seems to occur for an aberrant developmental program rather than impaired proliferation. LSD1 orthologs in *Drosophila melanogaster* and *Caenorhabditis elegans* are expressed in the germline, and the *Drosophila* LSD1 mutants have a germline-specific phenotype [17,43,44]. Inactivation of the *Drosophila* LSD1, Su(var)3-3 (suppressor of variegation 3-3), a suppressor of heterochromatic silencing, leads to sterility and tissue defects. In these mutant animals changes of global H3K4me2/me1 levels and reduction of H3K9me in heterochromatic regions have been observed. Data reported by the authors suggest that H3K4me2 LSD1 demethylase activity indirectly affects H3K9me levels. The *C. elegans* SPR-5 (suppressor of presenilin defect) protein has also been implicated in the control of H3K4me2 levels in the germline. Data reported by the authors suggest that H3K4me2 LSD1 demethylase activity indirectly affects H3K9me levels. The *C. elegans* SPR-5 (suppressor of presenilin defect) protein has also been implicated in the control of H3K4me2 levels in the germline. Increased levels of H3K4me2 are only observed in late generations, correlating with deregulation of genes expressed in spermatogenesis and animal infertility. In summary, LSD1 is essential for mouse development, while the LSD1 orthologs in *C. elegans* and *Drosophila* are important players in the germline. Collectively, studies in different model systems suggest that perturbation of enzymes that regulate H3K4me and H3K9me, such as LSD1, leads to defects in many meiotic steps and it is essential for mouse development. In addition, several reports strongly indicate that LSD1 is required for differentiation of several cell types [45], such as adipogenesis [46], skeletal muscle differentiation [47] and it is involved in the epithelial-to-mesenchymal transition. Epithelial to mesenchymal transition, EMT, is a paradigm of cell plasticity that it is characterized by reversible loss of epithelial characteristics and acquisition of mesenchymal properties. During EMT transition a global H3K9me2 reduction and H3K4me3 and H3K36me3 increase have been observed [48]. These changes have been found to be largely dependent on LSD1 activity and loss of LSD1 function has marked effects on EMT-driven cell migration and chemoresistance. In human cancer EMT is largely due to Snail mediated repression of transcription of epithelial genes and it has been shown that LSD1 functionally interacts with Snail and it is recruited on epithelial gene promoters [49]. In summary, the number of studies reporting a causative role of LSD1 in differentiation process in several different cell systems is currently growing.

5. Stemness control by LSD1

The pluripotent state of embryonic stem cells (ESCs) is of great interest as model system for studying the mechanisms controlling “stemness” such as self-renewal and pluripotency. It has been

hypothesized that the similarities shared by stem and cancer cells might be related to shared gene expression patterns. The acquisition of pluripotency during the reprogramming process is somehow reminiscent of the de-differentiation process resulting in cancers as proposed [50]. Intriguingly, it has been shown that the signaling pathways involved in the regulation of the biology of stem and cancer cells belong to functionally separable modules (Core, Polycomb and Myc) [51–53]. The pluripotent state of ESC is maintained through the combinatorial actions of core transcription factors, including Oct4, Sox2, and Nanog [53,54], microRNAs [55], signaling pathways [56,57], and most importantly regulatory mechanisms involving epigenetic regulation.

Recently, several reports correlate LSD1 function to the control of gene expression during ESC differentiation. It has been reported that LSD1 knock out in mouse ESCs shows increased apoptosis, impaired differentiation ability and, more importantly, failure to maintain the global levels of DNA methylation [41]. The same group described a mechanism that involves LSD1 in the regulation of Dnmt1. The demethylase Dnmt1 is a LSD1 substrate that removes K1096 residue of Dnmt1 resulting in the stabilization of the protein [41]. Thus, LSD1 and KMTs (Set7/9 responsible for Dnmt1 K1096 methylation) dynamically regulate the methylation status of Dnmt1 having a crucial role in development and differentiation through regulation of global DNA methylation. In another study, conditional LSD1 knockout in mouse ESCs does not affect their stem cell phenotype (i.e. expression of Oct4, Nanog, and alkaline phosphatase) thus indicating that LSD1 is not essential for the maintenance of ESC state [42]. ESC lacking LSD1 proliferate normally but show increased levels of cell death upon differentiation. Loss of LSD1 activity results in the premature activation of *brachyury*, a key regulator of mesoderm formation [42]. Additional evidences that the loss of LSD1 causes a precocious expression of developmental markers have been provided by Adamo et al., in human ESC. LSD1 knockdown by shRNA, in human ESC causes a decrease in growth rate characterized by partial cell arrest in the G0/G1 phase [58,59] and up-regulation of genes involved in the development processes such as FOXA2 (forkhead box A2), EOMES (eomesodermin), BMP2 (bone morphogenetic protein 2) and SOX17. These data suggest that LSD1 is essential to regulate balance between H3K4me2/me3 and H3K27me3 at target developmental genes that are poised in human ESC cells.

A study from Young's laboratory has recently provided further insight on the role of LSD1 in ESC differentiation. Using chromatin immunoprecipitation coupled with massive parallel DNA sequencing (ChIP-Seq), it has been shown that LSD1 occupies the enhancer and core promoters of a significant number of actively transcribed genes and that the enzyme is required during differentiation for enhancer silencing (enhancer decommissioning) of ESC specific genes mediated by the NuRD complex [60]. Upon differentiation, LSD1 demethylates H3K4me1 at the enhancer of ESC specific genes, thus determining repression of Oct4/Sox/Nanog-regulated gene targets. A mechanistic model for LSD1 function in ESC has been proposed. LSD1–NuRD complex binds to Oct4-active enhancers in ESCs but it is unable to demethylate H3K4. Since LSD1 activity is inhibited in the presence of acetylated histones [21,23], histone acetylation levels at enhancers co-occupied by both HATs and NuRD associated HDACs suppress demethylase activity of bound LSD1. During ESC differentiation, histone acetylation levels at ESC genes decrease, thus allowing LSD1-dependent demethylase activity, and then, gene silencing. These findings suggest a possible role of LSD1 in normal differentiation of ESCs, when the active enhancers must be silenced, rather than in the maintenance of the ESC state [60].

6. Myc and LSD1 co-occupancy in ES cells

Myc family members are critical to maintain embryonic stem cells (ESCs) in the undifferentiated state. Myc over expression can block differentiation and cooperates with Oct3/4, Sox2 and Klf4 to reprogram adult differentiated cells into induced pluripotent stem cells (iPS) that resemble ESC cells [61,62]. In mouse ESC, Myc regulates the transcription

of all core components of the Polycomb repressive complex 2 (PRC2) by binding to the E-box elements of PRC2 genes [63]. As discussed above, ChIP-Seq data revealed that LSD1 occupies the enhancer/promoter regions of actively transcribed genes in mouse ESCs [60]. Intriguingly, a large fraction of Myc targets correlate with LSD1 and Pol II occupancy. Fig. 2 shows Pol II, LSD1 and c-Myc binding to 5 Myc targets (Ncl, Apex1, Ezh2, Eed, Suz12). The functional relevance of the co-occupancy of Myc/LSD1/Pol II at Myc promoter targets in ES cells remains to be addressed, taking into account that it has been shown that recruitment of Myc/LSD1 on promoters is a necessary and early event to initiate transcription of Myc targets [32,33]. Chemical LSD1 inhibitors have been successfully used to block the growth of ES cells as well as pluripotent carcinomas as teratomas, embryonic carcinoma and seminoma [10]; and it is likely that the same chemical compounds can be used to dissect the role of Myc–LSD1 in gene expression in ES cells.

7. LSD1 and cancer

Notwithstanding LSD1 has been reported to be associated with cancer only few years ago and it has been found to possess oncogenic properties in several cancers ranging from prostate [64], bladder [65], neuroblastomas [66], lung cancers [67] sarcomas and hepatocarcinomas [68]. LSD1 inhibition reduces or blocks cell growth in many of these tumors, while its over-expression has been found to contribute to human carcinogenesis through chromatin modification [69].

7.1. LSD1 and acute myeloid leukemia

During the last year two different groups pointed out the role of LSD1 in leukemia and provided preclinical proof of concept for using LSD1 pharmacological inhibitors to treat acute leukemia [70,71].

Acute leukemias are clonal disorders of hematopoiesis in which leukemic stem cells (LSCs) develop unlimited self-renewal capacity, enhanced proliferation and impaired hematopoietic differentiation programs. Recent advances in leukemia biology come from studies

that investigated on genetic and epigenetic abnormalities in leukemic cells [72].

Leukemic stem cells (LSCs) are a functionally defined multipotent entity that can undergo self-renewal, the origin of which has been the subject of considerable research in recent years. During normal developmental progression from stem cell to progenitors and then mature cells, mutations may potentially occur at any stage giving rise to a malignant entity. The role of LSD1 as essential regulator of LSC potential has been described in a mouse and human models of human MLL-AF9 leukemia [73]. The extent of LSD1 knockdown significantly correlated with loss of the LSC potential of AML cells through impairment of differentiation and apoptosis. Cells without active LSD1 are unable to form colonies (consistent with loss of LSC potential) exhibit differentiated cell morphology and are not able to cause leukemia when introduced into mice [70]. The causative role of LSD1 over-expression in AML leukemia has been substantiated by the concomitant results obtained in a cytogenetically distinct subtype of AML, the acute promyelocytic leukemia, APL, characterized by a translocation involving promyelocytic leukemia gene, PML and the retinoic acid receptor, RAR α genes. Therapy of this leukemia with pharmacological concentrations of all-trans-retinoic-acid (ATRA) promotes differentiation of leukemic cells, but some APL subtypes are resistant to ATRA. However, concomitant administration of ATRA and a pharmacological inhibitor of LSD1 (tranylcypromine, TCP) induce morphological and immunophenotypic differentiation of APML cells in vitro. The findings that LSD1 has a major role in AML progression push the efforts to understand the genetic program regulated by LSD1 [71]. LSD1 may contribute directly or indirectly to maintain expression of key transcription factors and chromatin modifying enzymes. Expression analysis suggests that LSD1 might regulate a subset of genes that activate the oncogenic program associated with MLL-AF9 leukemia and chromatin immunoprecipitation and next generation sequencing, (ChIP-Seq), showed that H3K4me2 increase is the only detectable change at MLL-AF9 promoters following LSD1 silencing. These results clearly demonstrate that in AML, demethylation by LSD1 is associated with activation of LSC associated oncogenic target genes [70]. Given the relevant findings regarding the role of LSD1 in sustaining the oncogenic gene program and the block of differentiation in MLL cells, the mechanisms by which LSD1 functions

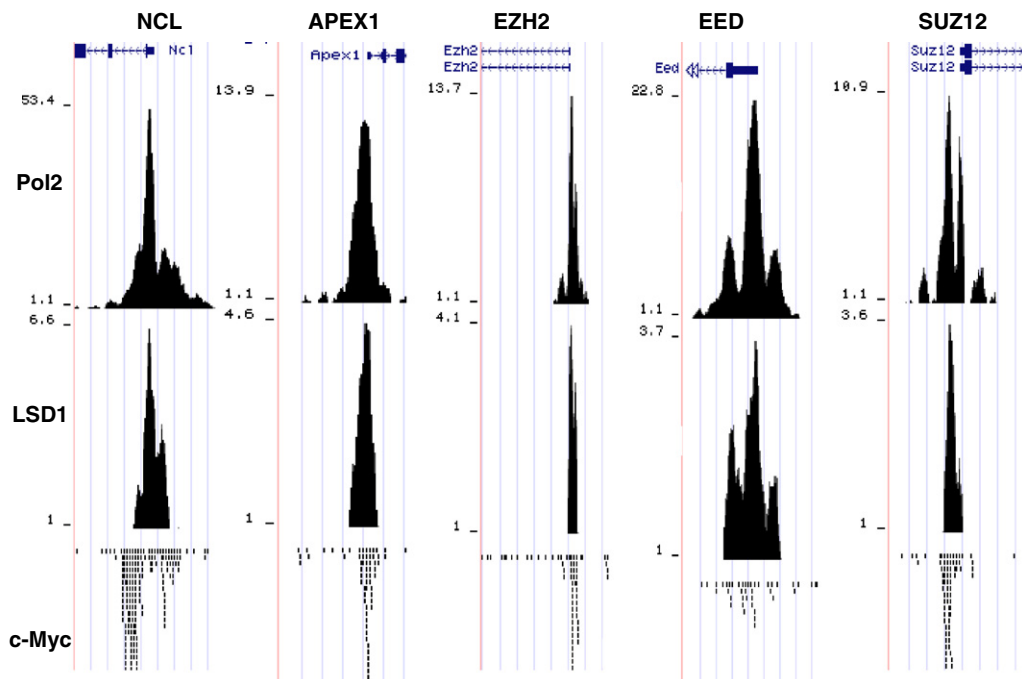


Fig. 2. An adapted UCSC Genome browser view displaying Pol II LSD1 and Myc binding on 5 Myc targets in mouse ES cells. Raw ChIP-Seq data were from GEO accession numbers: Pol2, GSE12680; LSD1, GSE27844; and c-Myc, GSE11431.

at its target genes and the protein complexes recruited by LSD1 at these loci require further investigations.

7.2. LSD1 and T cell leukemia

In T-cell acute lymphoblastic leukemia (T-ALL) resulting from the malignant transformation of T-cell progenitors, mutations in Notch1, leading to aberrant and constitutively active Notch1 signaling, contribute to oncogenic transformation and are hallmarks of this disease [74,75]. It has been recently found that LSD1 participates to a Notch-containing multifunctional complex; furthermore, together with the PFH8 demethylase it is involved in the epigenetic modification of Notch1 target genes [75]. In T-ALL, Notch mediated regulation of its targets is the first example of the dual role of LSD1 as activator or repressor; it is known that the DNA-binding factor CSL binds and represses Notch targets in the absence of Notch, while the presence of Notch converts CSL in a transcriptional activator. The finding that LSD1 interacts with CSL explains the mechanism through which Notch determines gene repression by removing the H3K4me2 marks at Notch targets in the absence of Notch. Indeed, a functional switch of LSD1 activity is observed upon Notch activation. In the absence of Notch, LSD1 triggers H3K4me2 demethylation while in its presence the enzyme acts preferentially on H3K9me2 leading to activation of target genes. Thus, LSD1 inhibition in T-ALL reproduces cell growth arrest and alteration of growth, and a phenotype was previously attributed to Notch silencing [75].

Huang's laboratory has shown that LSD1 is associated with the hematopoietic specific transcription factor TAL1/SCL whose dysregulation has been associated with T cell leukemogenesis. LSD1/Tal1 association is disrupted by phosphorylation of serine 172 in TAL1 by protein-kinase-A (PKA) and the destabilized TAL1–LSD1 interaction leads to promoter H3K4 hypermethylation and activation of target genes [76,77]. Thus, a PKA-dependent dynamic interaction between LSD1 and TAL1 has a causative role in hematopoiesis and leukemogenesis.

7.3. LSD1 and solid tumors

High levels of LSD1 protein have been found in several types of solid tumors and are associated with poor prognosis. This is the reason why LSD1 has been proposed as a biomarker for neuroblastoma, non-small cell lung carcinomas (NSCLC), breast and prostate cancers [65–69]. In NSCLC it has been shown that down regulation of LSD1 expression by the pharmacological inhibitor pargyline or by specific siRNAs determines suppression of cell growth, migration and invasion. Similarly, LSD1 immuno-reactivity is increased in a substantial fraction of hepatocarcinomas, (HCC), and LSD1 knockdown in HCC cells decreases substantial cell proliferation [68].

8. Conclusion and future directions

LSD1 is promiscuously bound at multiple sites across the genome predominantly at active promoters and enhancers. However, several different LSD1-containing complexes have been described; it is likely that the formation and stability of LSD1 complexes present on different gene targets depend on the cellular context and environmental signals. The major future challenge is the dissection of LSD1 epigenetic role and the mechanism of formation of these inhibitory or stimulatory LSD1 containing complexes. Further analysis to identify molecular signaling affecting the nature and function of LSD1-complexes will improve our knowledge of epigenetic modifications and their functional outcome in cancer and stem cell differentiation and reprogramming. Considering the association between high levels of LSD1 and neoplastic progression, LSD1 has become an attracting target for drug discovery, and it has been proposed that epigenetic drugs targeting LSD1 could be therapeutically used alone or in combination with other therapeutic tools. Recently, two reports have independently provided preclinical

proof of concept for using lysine-specific histone demethylase 1 inhibitors to treat acute myelogenous leukemia [70,71]. The discovery and development of flexible and selective LSD1 inhibitors will undoubtedly help our understanding of its role in epigenetic regulation of both in cancer and cell differentiation, and will allow the development of molecular therapies aimed to inhibit tumor growth and promote cell differentiation of tumor cells.

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References

- [1] R.A. Young, Control of the embryonic stem cell state, *Cell* 144 (2011) 940–954.
- [2] S.H. Orkin, K. Hochedlinger, Chromatin connections to pluripotency and cellular reprogramming, *Cell* 145 (2011) 835–850.
- [3] M.A. Dawson, T. Kouzarides, Cancer epigenetics: from mechanism to therapy, *Cell* 150 (2012) 12–27.
- [4] Q. Hu, M.G. Rosenfeld, Epigenetic regulation of human embryonic stem cells, *Front. Genet.* 3 (2012) 238.
- [5] J.C. Black, C. Van Rechem, J.R. Whetstone, Histone lysine methylation dynamics: establishment, regulation, and biological impact, *Mol. Cell* 48 (2012) 491–507.
- [6] E.L. Greer, Y. Shi, Histone methylation: a dynamic mark in health, disease and inheritance, *Nat. Rev. Genet.* 13 (2012) 343–357.
- [7] S. Sharma, T.K. Kelly, P.A. Jones, Epigenetics in cancer, *Carcinogenesis* 31 (2010) 27–36.
- [8] D. Rotili, A. Mai, Targeting histone demethylases: a new avenue for the fight against cancer, *Genes Cancer* 2 (2011) 663–679.
- [9] Y. Shi, Histone lysine demethylases: emerging roles in development, physiology and disease, *Nat. Rev. Genet.* 8 (2007) 829–833.
- [10] J.T. Lynch, W.J. Harris, T.C. Somerville, LSD1 inhibition: a therapeutic strategy in cancer? *Expert Opin. Ther. Targets* 16 (2012) 1239–1249.
- [11] I. Cohen, E. Poreba, K. Kamieniarz, R. Schneider, Histone modifiers in cancer: friends or foes? *Genes Cancer* 2 (2011) 631–647.
- [12] G.W. Humphrey, Y. Wang, V.R. Russanova, T. Hirai, J. Qin, Y. Nakatani, B.H. Howard, Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1, *J. Biol. Chem.* 276 (2001) 6817–6824.
- [13] Y. Shi, J. Sawada, G. Sui, B. Affar el, J.R. Whetstone, F. Lan, H. Ogawa, M.P. Luke, Y. Nakatani, Coordinated histone modifications mediated by a CtBP co-repressor complex, *Nature* 422 (2003) 735–738.
- [14] Y. Shi, F. Lan, C. Matson, P. Mulligan, J.R. Whetstone, P.A. Cole, R.A. Casero, Histone demethylation mediated by the nuclear amine oxidase homolog LSD1, *Cell* 119 (2004) 941–953.
- [15] A. Karytinov, F. Forneris, A. Profumo, G. Ciossani, E. Battaglioli, C. Binda, A. Mattevi, A novel mammalian flavin-dependent histone demethylase, *J. Biol. Chem.* 284 (2009) 17775–17782.
- [16] M. Opel, D. Lando, C. Bonilla, S.C. Treweek, A. Boukaba, J. Walfridsson, J. Cauwood, P.J. Werler, A.M. Carr, T. Kouzarides, N.V. Murzina, R.C. Allshire, K. Ekwall, E.D. Laue, Genome-wide studies of histone demethylation catalysed by the fission yeast homologues of mammalian LSD1, *PLoS One* 2 (2007) e386.
- [17] T. Rudolph, M. Yonezawa, S. Lein, K. Heidrich, S. Kubicek, C. Schafer, S. Phalke, M. Walther, A. Schmidt, T. Jenuwein, G. Reuter, Heterochromatin formation in *Drosophila* is initiated through active removal of H3K4 methylation by the LSD1 homolog SU(VAR)3–3, *Mol. Cell* 26 (2007) 103–115.
- [18] F. Forneris, C. Binda, M.A. Vanoni, A. Mattevi, E. Battaglioli, Histone demethylation catalysed by LSD1 is a flavin-dependent oxidative process, *FEBS Lett.* 579 (2005) 2203–2207.
- [19] F. Forneris, C. Binda, E. Battaglioli, A. Mattevi, LSD1: oxidative chemistry for multifaceted functions in chromatin regulation, *Trends Biochem. Sci.* 33 (2008) 181–189.
- [20] F. Lan, A.C. Nottke, Y. Shi, Mechanisms involved in the regulation of histone lysine demethylases, *Curr. Opin. Cell Biol.* 20 (2008) 316–325.
- [21] F. Forneris, C. Binda, M.A. Vanoni, E. Battaglioli, A. Mattevi, Human histone demethylase LSD1 reads the histone code, *J. Biol. Chem.* 280 (2005) 41360–41365.
- [22] Y. Chen, Y. Yang, F. Wang, K. Wan, K. Yamane, Y. Zhang, M. Lei, Crystal structure of human histone lysine-specific demethylase 1 (LSD1), *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 13956–13961.
- [23] M.G. Lee, C. Wynder, D.M. Schmidt, D.G. McCafferty, R. Shiekhattar, Histone H3 lysine 4 demethylation is a target of nonselective antidepressive medications, *Chem. Biol.* 13 (2006) 563–567.
- [24] C. Binda, S. Valente, M. Romanenghi, S. Pilotto, R. Cirilli, A. Karytinov, G. Ciossani, O.A. Botrugno, F. Forneris, M. Tardugno, D.E. Edmondson, S. Minucci, A. Mattevi, A. Mai, Biochemical, structural, and biological evaluation of tranlycypromine derivatives as inhibitors of histone demethylases LSD1 and LSD2, *J. Am. Chem. Soc.* 132 (2010) 6827–6833.

- [25] J.C. Culhane, D. Wang, P.M. Yen, P.A. Cole, Comparative analysis of small molecules and histone substrate analogues as LSD1 lysine demethylase inhibitors, *J. Am. Chem. Soc.* 132 (2010).
- [26] E. Metzger, M. Wissmann, N. Yin, J.M. Muller, R. Schneider, A.H. Peters, T. Gunther, R. Buettner, R. Schule, LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription, *Nature* 437 (2005) 436–439.
- [27] M. Wissmann, N. Yin, J.M. Muller, H. Greschik, B.D. Fodor, T. Jenuwein, C. Vogler, R. Schneider, T. Gunther, R. Buettner, E. Metzger, R. Schule, Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression, *Nat. Cell Biol.* 9 (2007) 347–353.
- [28] B. Perillo, M.N. Ombra, A. Bertoni, C. Cuzzo, S. Sacchetti, A. Sasso, L. Chiariotti, A. Malorni, C. Abbondanza, E.V. Avvedimento, DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression, *Science* 319 (2008) 202–206.
- [29] I. Garcia-Bassets, Y.S. Kwon, F. Telese, G.G. Prefontaine, K.R. Hutt, C.S. Cheng, B.G. Ju, K.A. Ohgi, J. Wang, L. Escoubet-Lozach, D.W. Rose, C.K. Glass, X.D. Fu, M.G. Rosenfeld, Histone methylation-dependent mechanisms impose ligand dependency for gene activation by nuclear receptors, *Cell* 128 (2007) 505–518.
- [30] E. Metzger, A. Imhof, D. Patel, P. Kahl, K. Hoffmeyer, N. Friedrichs, J.M. Muller, H. Greschik, J. Kirfel, S. Ji, N. Kunowska, C. Beisenherz-Huss, T. Gunther, R. Buettner, R. Schule, Phosphorylation of histone H3T6 by PKCbeta(1) controls demethylation at histone H3K4, *Nature* 464 (2010) 792–796.
- [31] S.S. Nair, B.C. Nair, V. Cortez, D. Chakravarty, E. Metzger, R. Schule, D.W. Brann, R.R. Tekmal, R.K. Vadlamudi, PELP1 is a reader of histone H3 methylation that facilitates oestrogen receptor-alpha target gene activation by regulating lysine demethylase 1 specificity, *EMBO Rep.* 11 (2010) 438–444.
- [32] S. Amente, A. Bertoni, A. Morano, L. Lania, E.V. Avvedimento, B. Majello, LSD1-mediated demethylation of histone H3 lysine 4 triggers Myc-induced transcription, *Oncogene* 29 (2010) 3691–3702.
- [33] S. Amente, L. Lania, E.V. Avvedimento, B. Majello, DNA oxidation drives Myc mediated transcription, *Cell Cycle* 9 (2010) 3002–3004.
- [34] D. Biswas, T.A. Milne, V. Basur, J. Kim, K.S. Elenitoba-Johnson, C.D. Allis, R.G. Roeder, Function of leukemogenic mixed lineage leukemia 1 (MLL) fusion proteins through distinct partner protein complexes, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 15751–15756.
- [35] T. Nakamura, T. Mori, S. Tada, W. Krajewski, T. Rozovskaia, R. Wassell, G. Dubois, A. Mazo, C.M. Croce, E. Canaani, ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation, *Mol. Cell* 10 (2002) 1119–1128.
- [36] J. Huang, R. Sengupta, A.B. Espejo, M.G. Lee, J.A. Dorsey, M. Richter, S. Opravil, R. Shiekhattar, M.T. Bedford, T. Jenuwein, S.L. Berger, p53 is regulated by the lysine demethylase LSD1, *Nature* 449 (2007) 105–108.
- [37] A. Scoumanne, X. Chen, The lysine-specific demethylase 1 is required for cell proliferation in both p53-dependent and -independent manners, *J. Biol. Chem.* 282 (2007) 15471–15475.
- [38] H. Kontaki, I. Talianidis, Cross-talk between post-translational modifications regulate life or death decisions by E2F1, *Cell Cycle* 9 (2010) 3836–3837.
- [39] H. Kontaki, I. Talianidis, Lysine methylation regulates E2F1-induced cell death, *Mol. Cell* 39 (2010) 152–160.
- [40] H.S. Cho, T. Suzuki, N. Dohmae, S. Hayami, M. Unoki, M. Yoshimatsu, G. Toyokawa, M. Takawa, T. Chen, J.K. Kurash, H.J. Field, B.A. Ponder, Y. Nakamura, R. Hamamoto, Demethylation of RB regulator MYPT1 by histone demethylase LSD1 promotes cell cycle progression in cancer cells, *Cancer Res.* 71 (2011) 655–660.
- [41] J. Wang, S. Hevi, J.K. Kurash, H. Lei, F. Gay, J. Bajko, H. Su, W. Sun, H. Chang, G. Xu, F. Gaudet, E. Li, T. Chen, The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation, *Nat. Genet.* 41 (2009) 125–129.
- [42] C.T. Foster, O.M. Dovey, L. Lezina, J.L. Luo, T.W. Gant, N. Barlev, A. Bradley, S.M. Cowley, Lysine-specific demethylase 1 regulates the embryonic transcriptome and CoREST stability, *Mol. Cell. Biol.* 30 (2010) 4851–4863.
- [43] L. Di Stefano, J.Y. Ji, N.S. Moon, A. Herr, N. Dyson, Mutation of *Drosophila* Lsd1 disrupts H3-K4 methylation, resulting in tissue-specific defects during development, *Curr. Biol.* 17 (2007) 808–812.
- [44] D.J. Katz, T.M. Edwards, V. Reinke, W.G. Kelly, A. C. *elegans* LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory, *Cell* 137 (2009) 308–320.
- [45] J. Wang, K. Scully, X. Zhu, L. Cai, J. Zhang, G.G. Prefontaine, A. Krones, K.A. Ohgi, P. Zhu, I. Garcia-Bassets, F. Liu, H. Taylor, J. Lozach, F.L. Jayes, K.S. Korach, C.K. Glass, X.D. Fu, M.G. Rosenfeld, Opposing LSD1 complexes function in developmental gene activation and repression programmes, *Nature* 446 (2007) 882–887.
- [46] M.M. Musri, C. Carmona, F.A. Hanzu, P. Kaliman, R. Gomis, M. Parrizas, Histone demethylase LSD1 regulates adipogenesis, *J. Biol. Chem.* 285 (2010) 30034–30041.
- [47] J. Choi, H. Jang, H. Kim, S.T. Kim, E.J. Cho, H.D. Youn, Histone demethylase LSD1 is required to induce skeletal muscle differentiation by regulating myogenic factors, *Biochem. Biophys. Res. Commun.* 401 (2010) 327–332.
- [48] O.G. McDonald, H. Wu, W. Timp, A. Doi, A.P. Feinberg, Genome-scale epigenetic reprogramming during epithelial-to-mesenchymal transition, *Nat. Struct. Mol. Biol.* 18 (2011) 867–874.
- [49] T. Lin, A. Ponn, X. Hu, B.K. Law, J. Lu, Requirement of the histone demethylase LSD1 in Snail1-mediated transcriptional repression during epithelial–mesenchymal transition, *Oncogene* 29 (2010) 4896–4904.
- [50] J. Kim, S.H. Orkin, Embryonic stem cell-specific signatures in cancer: insights into genomic regulatory networks and implications for medicine, *Genome Med.* 3 (2011) 75.
- [51] L.A. Boyer, T.I. Lee, M.F. Cole, S.E. Johnstone, S.S. Levine, J.P. Zuckerman, M.G. Guenther, R.M. Kumar, H.L. Murray, R.G. Jenner, D.K. Gifford, D.A. Melton, R. Jaenisch, R.A. Young, Core transcriptional regulatory circuitry in human embryonic stem cells, *Cell* 122 (2005) 947–956.
- [52] X. Chen, H. Xu, P. Yuan, F. Fang, M. Huss, V.B. Vega, E. Wong, Y.L. Orlov, W. Zhang, J. Jiang, Y.H. Loh, H.C. Yeo, Z.X. Yeo, V. Narang, K.R. Govindarajan, B. Leong, A. Shahab, Y. Ruan, G. Bourque, W.K. Sung, N.D. Clarke, C.L. Wei, H.H. Ng, Integration of external signaling pathways with the core transcriptional network in embryonic stem cells, *Cell* 133 (2008) 1106–1117.
- [53] J. Kim, J. Chu, X. Shen, J. Wang, S.H. Orkin, An extended transcriptional network for pluripotency of embryonic stem cells, *Cell* 132 (2008) 1049–1061.
- [54] Y.H. Loh, Q. Wu, J.L. Chew, V.B. Vega, W. Zhang, X. Chen, G. Bourque, J. George, B. Leong, J. Liu, K.Y. Wong, K.W. Sung, C.W. Lee, X.D. Zhao, K.P. Chiu, L. Lipovich, V.A. Kuznetsov, P. Robson, L.W. Stanton, C.L. Wei, Y. Ruan, B. Lim, H.H. Ng, The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells, *Nat. Genet.* 38 (2006) 431–440.
- [55] A. Marson, S.S. Levine, M.F. Cole, G.M. Frampton, T. Brambrink, S. Johnstone, M.G. Guenther, W.K. Johnston, M. Wernig, J. Newman, J.M. Calabrese, L.M. Dennis, T.L. Volkert, S. Gupta, J. Love, N. Hannett, P.A. Sharp, D.P. Bartel, R. Jaenisch, R.A. Young, Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells, *Cell* 134 (2008) 521–533.
- [56] H. Niwa, T. Burdon, I. Chambers, A. Smith, Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3, *Genes Dev.* 12 (1998) 2048–2060.
- [57] N. Sato, L. Meijer, L. Skaltsounis, P. Greengard, A.H. Brivanlou, Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor, *Nat. Med.* 10 (2004) 55–63.
- [58] A. Adamo, M.J. Barrero, J.C. Izpisua Belmonte, LSD1 and pluripotency: a new player in the network, *Cell Cycle* 10 (2011) 3215–3216.
- [59] A. Adamo, B. Sese, S. Boue, J. Castano, I. Paramonov, M.J. Barrero, J.C. Izpisua Belmonte, LSD1 regulates the balance between self-renewal and differentiation in human embryonic stem cells, *Nat. Cell Biol.* 13 (2011) 652–659.
- [60] W.A. Whyte, S. Bilodeau, D.A. Orlando, H.A. Hoke, G.M. Frampton, C.T. Foster, S.M. Cowley, R.A. Young, Enhancer decommissioning by LSD1 during embryonic stem cell differentiation, *Nature* 482 (2012) 221–225.
- [61] P. Cartwright, C. McLean, A. Sheppard, D. Rivett, K. Jones, S. Dalton, LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism, *Development* 132 (2005) 885–896.
- [62] N.V. Varlakhanova, R.F. Cotterman, W.N. deVries, J. Morgan, L.R. Donahue, S. Murray, B.B. Knowles, P.S. Knoepfler, myc maintains embryonic stem cell pluripotency and self-renewal, *Differentiation* 80 (2010) 9–19.
- [63] F. Neri, A. Zippo, A. Krepelova, A. Cherubini, M. Rocchigiani, S. Oliviero, Myc regulates the transcription of the PRC2 gene to control the expression of developmental genes in embryonic stem cells, *Mol. Cell. Biol.* 32 (2012) 840–851.
- [64] P. Kahl, L. Gullotti, L.C. Heukamp, S. Wolf, N. Friedrichs, R. Vorreuther, G. Solleder, P.J. Bastian, J. Ellinger, E. Metzger, R. Schule, R. Buettner, Androgen receptor coactivators lysine-specific histone demethylase 1 and four and a half LIM domain protein 2 predict risk of prostate cancer recurrence, *Cancer Res.* 66 (2006) 11341–11347.
- [65] E.C. Kauffman, B.D. Robinson, M.J. Downes, L.G. Powell, M.M. Lee, D.S. Scherr, L.J. Gudas, N.P. Mongan, Role of androgen receptor and associated lysine-demethylase coregulators, LSD1 and JMJD2A, in localized and advanced human bladder cancer, *Mol. Carcinog.* 50 (2011) 931–944.
- [66] J.H. Schulte, S. Lim, A. Schramm, N. Friedrichs, J. Koster, R. Versteeg, I. Ora, K. Pajtlar, L. Klein-Hitpass, S. Kuhfittig-Kulle, E. Metzger, R. Schule, A. Eggert, R. Buettner, J. Kirfel, Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy, *Cancer Res.* 69 (2009) 2065–2071.
- [67] T. Lv, D. Yuan, X. Miao, Y. Lv, P. Zhan, X. Shen, Y. Song, Over-expression of LSD1 promotes proliferation, migration and invasion in non-small cell lung cancer, *PLoS One* 7 (2012) e35065.
- [68] Z.K. Zhao, P. Dong, J. Gu, L. Chen, M. Zhuang, W.J. Lu, D.R. Wang, Y.B. Liu, Overexpression of LSD1 in hepatocellular carcinoma: a latent target for the diagnosis and therapy of hepatoma, *Tumour Biol.* (2012).
- [69] S. Hayami, J.D. Kelly, H.S. Cho, M. Yoshimatsu, M. Unoki, T. Tsunoda, H.I. Field, D.E. Neal, H. Yamaue, B.A. Ponder, Y. Nakamura, R. Hamamoto, Overexpression of LSD1 contributes to human carcinogenesis through chromatin regulation in various cancers, *Int. J. Cancer* 128 (2011) 574–586.
- [70] W.J. Harris, X. Huang, J.T. Lynch, G.J. Spencer, J.R. Hitchin, Y. Li, F. Ciceri, J.G. Blaser, B.F. Greystoke, A.M. Jordan, C.J. Miller, D.J. Oglivie, T.C. Somerville, The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells, *Cancer Cell* 21 (2012) 473–487.
- [71] T. Schenk, W.C. Chen, S. Gollner, L. Howell, L. Jin, K. Hebestreit, H.U. Klein, A.C. Popescu, A. Burnett, K. Mills, R.A. Casero Jr., L. Marton, P. Woster, M.D. Minden, M. Dugas, J.C. Wang, J.E. Dick, C. Muller-Tidow, K. Petrie, A. Zelen, Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-trans-retinoic acid differentiation pathway in acute myeloid leukemia, *Nat. Med.* 18 (2012) 605–611.
- [72] L.P. Vu, L. Luciani, S.D. Nimer, Histone-modifying enzymes: their role in the pathogenesis of acute leukemia and their therapeutic potential, *Int. J. Hematol.* (2013).
- [73] A.A. Lokken, N.J. Zeleznik-Le, Breaking the LSD1/KDM1A addiction: therapeutic targeting of the epigenetic modifier in AML, *Cancer Cell* 21 (2012) 451–453.
- [74] C. Lobry, P. Oh, I. Aifantis, Oncogenic and tumor suppressor functions of Notch in cancer: it's NOTCH what you think, *J. Exp. Med.* 208 (2011) 1931–1935.
- [75] A. Yatim, C. Benne, B. Sobhian, S. Laurent-Chaballier, O. Deas, J.G. Judde, J.D. Lelievre, Y. Levy, M. Benkirane, NOTCH1 nuclear interactome reveals key regulators of its transcriptional activity and oncogenic function, *Mol. Cell* 48 (2012) 445–458.
- [76] X. Hu, X. Li, K. Valverde, X. Fu, C. Noguchi, Y. Qiu, S. Huang, LSD1-mediated epigenetic modification is required for TAL1 function and hematopoiesis, *Proc. Natl. Acad. Sci. USA* 106 (2009) 10141–10146.
- [77] Y. Li, C. Deng, X. Hu, B. Patel, X. Fu, Y. Qiu, M. Brand, K. Zhao, S. Huang, Dynamic interaction between TAL1 oncoprotein and LSD1 regulates TAL1 function in hematopoiesis and leukemogenesis, *Oncogene* 31 (2012) 5007–5018.